

# RELATIONSHIP BETWEEN BARNACLE EPIBIOTIC LOAD AND HEMATOLOGIC PARAMETERS IN LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*), A COMPARISON BETWEEN MIGRATORY AND RESIDENTIAL ANIMALS IN PAMLICO SOUND, NORTH CAROLINA

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**Abstract:** Health status of a total of 57 loggerhead sea turtles (*Caretta caretta*; 42 migratory and 15 residential turtles) was analyzed using body condition and hematologic parameters. A subset of 18 juvenile migratory loggerhead sea turtles in the fall of 1997 and 15 residential turtles in the summer of 2000 were analyzed for barnacle epibiota. The migratory group had significantly higher red blood cell counts and percent heterophils and significantly lower percent lymphocyte and absolute eosinophil counts, as well as significantly lower plasma concentrations of calcium, sodium, chloride, potassium, glucose, alkaline phosphatase, and anion gap. Many of these variations may be because of physiology of migration. A positive association between turtle weight and hematocrit was detected and may be because of larger turtles diving for longer periods of time. There were no significant differences of epibiota load, health of the turtles, or condition index between turtles captured during the two events.

**Key words:** barnacle, *Caretta caretta*, epibiota, loggerhead sea turtle, health, hematology, body condition, migration.

## INTRODUCTION

Sea turtles are the only reptiles that migrate long distances, and migration has been demonstrated to have significant physiologic effects on many mammal, bird, fish and invertebrate species.<sup>11</sup> These effects can influence the health status of animals during extensive migrations. Through these long migrations, the turtle encounters many changes, including possible physiologic stresses; changes in aspects of its environment such as temperature, current, light, and salinity; and animal populations including potential epibiotrophic organisms. Epibiota have been thought to be an external indicator of a turtle's health. Epibiotic load on sea turtle carapaces varies dramatically between individuals.

This may be because of individual characteristics such as growth (shell keratin turnover) and activity level, or because of environmental and location influences including prevalence of various epibiotic organisms, temperature, current, or salinity as well as many other factors. The accumulation of large epibiotic loads has been considered a potential marker of physical compromise of sea turtles, but few data are available to test this assumption. The value of epibiota estimations for assessing general health of sea turtle populations has not been fully explored. If epibiotic load assessments correlate with more specialized indicators of general health status such as hematologic parameters, plasma enzyme chemistries, and/or body condition indices, it may be a practical marker for sea turtle population health.

In the western Atlantic, the Chesapeake Bay is a major seasonal developmental habitat for 5,000–10,000 loggerhead sea turtles (*Caretta caretta*) present each summer. From late September to early November, juvenile loggerhead sea turtles migrate south, arriving at Cape Hatteras, North Carolina, around December. They are joined in the fall by substantial numbers of juvenile loggerheads (residential) from the sounds of North Carolina. By January, most turtles are south of Cape Hatteras.<sup>9</sup> Loggerhead sea turtles have been observed to emigrate offshore during the winter months.<sup>5</sup> The objective of this paper is to compare the health status of mi-

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gratory and residential animals in Pamlico and Core Sounds, North Carolina, and to evaluate the relationship between epibiotic barnacle loads on the carapace and accepted markers of loggerhead sea turtle general health status, including condition index, body weight, and hematologic and plasma chemistry values.

## MATERIALS AND METHODS

### Subjects and sample collection

All turtles were caught in pound net fisheries located in Pamlico and Core Sounds, North Carolina (35°2.41'N, 76°7.13'W, 34°58.8'N, 76°12.9'W, 34°56.03'N 76°15.95'W). The pound nets were fished several times a week; thus, turtles were trapped in the pound nets no longer than 2 or 3 days. These turtles had an adequate food source and could freely surface to breathe while in confinement. All animals captured were juvenile loggerhead turtles with standard straight carapace lengths between 50 and 70 cm. Animals were caught in November 1997 (migratory turtles;  $n = 42$  turtles) and August of 2000 (resident turtles;  $n = 15$ ). Residential status was further defined as turtles that had been recaptured within Core and/or Pamlico Sound two or more times between May and September over a 5-yr period. Migratory turtles were defined as animals caught during established movements of large numbers of turtles occurring from October through December. Mean water temperature during the summer capture was 28°C, with  $SD \pm 0.2^\circ C$ . Fall captures water had a mean temperature of 14.5°C with  $SD 1.1^\circ C$ .

### Blood sample analysis

Blood was analyzed from 42 migratory turtles and 15 resident turtles. Approximately 15 ml of blood was taken within 15 min of capture from the net to assess health status. Blood was stored in heparin tubes (Monoject®, Sherwood Medical, St. Louis, Missouri 63103, USA) for CBC count and plasma chemistries. Blood samples were processed within 6 hr. Plasma was collected by centrifugation, placed in polyethylene cryogenic vials (Nalgene® Cryoware™, Nalge Company, Rochester, New York 14602-0265, U.S.A.) via micropipette, and stored at  $-70^\circ C$  until plasma chemistry analysis was completed within 1 week. Natt-Herrick solution and Neubauer counting chambers (American Optical Corp., Scientific Instrument Div., Buffalo, New York 14215, USA) were used to obtain red and white blood cell counts (RBC and WBC) utilizing previously reported techniques.<sup>2</sup> Differential counts were performed using Wright-Geimsa-stained thin

blood smears to determine percentage of total leucocytes and absolute counts of heterophils (HET), lymphocytes (LYMPH), azurophils (AZURO), eosinophils (EOS), and basophils. Hematocrit (HCT) was obtained by measuring the percentage of packed cells in the total volume of blood spun in a glass HCT tube using a centrifuge (Readacrit®, Clay Adams, Becton, Dickinson and Company, Parsippany, New Jersey 07054, USA). Plasma chemistry analyses were performed on an automated clinical analyzer (Roche Diagnostic Hitachi 912, Indianapolis, Indiana 46256, USA) that returned concentrations of calcium (Ca), phosphorus (P), aspartate aminotransferase (AST), sodium (Na), potassium (K), chloride (Cl), blood urea nitrogen (BUN), uric acid (UA), glucose (GLU), creatinine, total protein (TP), albumin (ALB), globulin (GLOB), alkaline phosphatase (ALP), magnesium, lactate dehydrogenase (LDH), total bilirubin, and gamma glutamyltransferase (GGT). Blood films were digitally photographed at a magnification of 400 $\times$  and red blood cell surface areas were calculated using Adobe Photoshop® (Adobe Systems Inc. San Jose, California 95110-2704, USA). Because different centrifuges of the same manufacturer were used on this project, a comparison of the centrifuge speed and time on hematocrit measurements was performed using two sea turtle blood samples; these were split and samples spun for 5 min with a variable speed centrifuge (Select-a-Fuge 24® Model 24-0224, Allen Medical Instrument Division, Bio-Dynamics, Inc. Santa Ana, California, USA). Graded settings were 4,000, 6,000, and 8,000 rpm. The same samples were run at 4,000 and 8,000 rpm for 3, 5, 7, and 10 min. An additional 4 samples were run for 5 min at 4,000, 5,000, 6,000, 7,000, 8,000 and 10,400 rpm on a separate centrifuge (Centrifuge 2, Triac Centrifuge®, Clay Adams Brand, Model 420200, Becton Dickinson Primary Care Diagnostics, Sparks, Maryland 21152, USA).

### Turtle characteristics

A physical examination was performed on each turtle; characteristics assessed included overall body condition impression, and epibiota identification and quantification, and any abnormalities were noted. Straight and curved carapace lengths were taken using forestry calipers (Haglof Inc., Madison, Wisconsin 39130, USA) and 120-cm vinyl coated fiberglass flat tailor's tape (The Perfect Measuring Tape Co. Toledo, Ohio 43604, USA). Length/weight and Fulton's K (weight/length<sup>3</sup>[W/L<sup>3</sup>]) were used to calculated body condition.<sup>8</sup> Laparoscopy using a 7-mm rigid laparoscope with a 50°

angle (Wolf Lumina®, Richard Wolf, Knittlingen, Germany) was performed to determine gender.<sup>13</sup> Local anesthesia consisted of 5 ml of 2% lidocaine (Phoenix Pharmaceuticals, Inc., St. Joseph, Missouri 64503, USA) placed in the inguinal area using a 5-ml syringe with a 20-ga needle. The area was surgically scrubbed using 5% povidone iodine (Betadine®, Purdue Frederick, Stamford, Connecticut 06901-3431, USA). Turtles were marked with both Inconel flipper tags (National Band and Tag Company, Newport, Kentucky 41072-0430, USA) and Passive Integrated Transponder (PIT) tags (AVID DNAchip®, AVIN United States, Norco, California 91760, USA) so that recaptures would be recognized.

### Barnacle study

Eighteen turtles in November and 15 turtles in August were photographed for the barnacle study. Only barnacles on the turtle's carapace were counted. A sample of all species of barnacle and other epifauna found were removed and placed in 70% ethanol for later identification. All turtles were photographed outdoors using a Pentax 105 IQ Zoom Point-And-Shoot Camera™ (Pentax Corporation, Englewood, Colorado 80112, USA) with a 38–105 mm Nikon AF Zoom Nikkor™ lens (Nikon Corporation, Chiyoda-Ku, Tokyo 100, Japan) and 100-speed color film (Kodak Gold™, Eastman Kodak Company, Rochester, New York 14650, USA) mounted on a tripod (ACME-Lite Manufacturing, Elk Grove Village, Illinois 60007, USA). To provide consistency, all photographs were taken from the dorsal ventral view with the camera between 45 and 50 cm from the carapace surface. A ruler was placed on each side of the carapace to provide a scale for reference when calculating areas using graphic analysis software. For the migratory cohort, the photographs were analyzed with Sigma-Scan™ software (SPSS Science, Chicago, Illinois 60606-6307, USA) using a digitizing tablet and cross hair cursor (Numonics Corporation, Montgomeryville, Pennsylvania 18936, USA). For the resident cohort, pictures were scanned in and saved as high-resolution JPEG files, which were then analyzed using Optimas 6.5 digital analysis software (Media Cybernetics, Silver Spring, Maryland 20910, USA). Five sets of pictures from the migratory cohort (L and R for each of 5 turtles) were analyzed with the Optimas system to calculate the percent coverage and validate the Optimas results with the SigmaPlot systems. Only the dominant species, *Chelonibia testudinaria*, was included in this analysis because it is sea turtle-specific and ubiquitous for loggerhead sea turtles.

### Calculation of carapace area and percent barnacle coverage

The carapace area and percent barnacle coverage for migratory turtles were determined by digital analysis using Sigma-Plot computer software (SPSS Inc., Chicago, Illinois 60606-6412, USA). The two photographs taken of each turtle were placed on a digitizing board and the Sigma-Plot program was calibrated with the scale included in each photograph. The digitizing board's pointer was then used manually to outline the perimeter of each barnacle and the half carapace in each picture, enabling Sigma-Plot to calculate the areas (cm<sup>2</sup>) enclosed within each outline. For resident turtles, each JPEG image was opened in Optimas and enlarged so that the barnacles could be seen more clearly. The area tool was then used to draw outlines around the perimeter of each barnacle, as well as the half of the carapace in each image, after which Optimas calculated the areas enclosed within those outlines. For all turtles, both total carapace area and total barnacle area were then calculated, making it possible to determine the percentage of the carapace area covered by barnacles. Barnacle counts (the total number of barnacles on each turtle) were also made. Dead barnacles were infrequently encountered but were included in the assessment because they covered carapace space.

### Statistics

Nonparametric statistical tests were used for comparisons because of the non-normal distribution of some data (Shapiro-Wilk test; JMP, SAS Institute, Cary, North Carolina 27513-2414, USA). Percentiles were set at 10th and 90th. To compare between groups, the Wilcoxon rank sum test (JMP) was used with the sequential Bonferroni method to reduce type I error.<sup>12</sup> The Kendall-tau test (JMP) was used for correlations.

## RESULTS

See Table 1 for summary results.

### Animals

The migratory loggerheads had a median (10th and 90th percentiles) standard straight carapace length of 59.5 cm (52.4–65.7 cm). Their median weight was 31.8 kg (21.6–41.2 kg) and their median body condition ( $W/L^3$ ) was 1.54 (1.35–1.74). Residential turtles had a mean straight carapace length of 64.6 cm (52.3–72.7 cm). Their median weight was 41 kg (23.8–52.8 kg) and their median body condition was 1.4 (1.34–1.92). There were 28 female and 14 male turtles in the migratory population, and seven females, six males, and two un-

Table 1. Resident versus migrant populations comparison of biological statistics.

	Migratory median	Migratory 10th and 90th percentiles	Resident median	Resident 10th and 90th percentiles	Wilcoxon rank sum test	Sequential Bonferroni test $P < 0.0015$
Weight kg	31.80	21.56–41.24	41.00	23.80–52.80	0.0059	
Length cm	59.45	52.35–65.70	64.55	52.25–72.70	0.0033	
W/L <sup>3a</sup> ratio	0.000154	0.000135–0.000174	0.000140	0.000134–0.000192	0.0651	
Barnacle coverage (%)	13.02	0.40–29.09	10.77	4.24–28.23	0.7468	
WBC, <sup>b</sup> 1,000/mm <sup>3</sup>	14.30	4.66–21.90	15.80	8.46–20.01	0.3651	
RBC, <sup>c</sup> 10 <sup>6</sup> /mm <sup>3</sup>	0.88	0.64–1.18	0.38	0.28–0.57	0.0001	significant
HCT <sup>d</sup>	28.0	23.0–35.6	32.0	23.6–36.8	0.0185	
%HET <sup>e</sup>	60.0	18.4–74.6	27.0	11.2–47.6	0.0002	significant
Heterophils/mm <sup>3</sup>	6.33	1.75–17.20	3.48	1.29–8.21	0.622	
Lymphocyte, %	36.0	17.4–64.8	65.0	42.8–82.4	0.0001	significant
Lymphocytes/mm <sup>3</sup>	4.29	1.56–9.36	9.92	4.84–13.51	0.0001	
Eosinophils, %	2.0	0.0–10.6	6.0	1.6–15.8	0.0058	
Eosinophils/mm <sup>3</sup>	0.23	0.0–1.47	0.88	0.21–2.55	0.0009	significant
Basophils, %	0.0	0.0–0.0	0.0	0.0–0.4	0.8496	
Basophils/mm <sup>3</sup>	0.0	0.0–0.0	0.0	0.0–0.15	0.8171	
Azuropihils, %	4.0	1.0–9.8	3.0	1.0–8.2	0.2443	
Azuropihils/mm <sup>3</sup>	1.52	0.0–4.38	0.75	0.23–1.40	0.0067	
Ca <sup>f</sup> mmol/l (mg/dl)	1.55 (6.20)	1.35–2.05 (5.38–8.18)	2.20 (8.80)	1.89–2.73 (7.56–10.92)	0.0001	significant
P <sup>g</sup> mmol/l (mg/dl)	2.03 (6.30)	1.57–2.45 (4.88–7.60)	2.26 (7.00)	1.85–2.70 (5.72–8.38)	0.0164	
CP <sup>h</sup> Ratio	1.00	0.74–1.47	1.29	0.91–1.83	0.0219	
AST, IU/L	129.0	81.4–179.0	206.0	129.2–329.2	0.0001	significant
Na <sup>i</sup> , mmol/l	151.0	148.0–156.2	158.0	154.0–159.0	0.0001	significant
K <sup>j</sup> , mmol/l	3.30	2.84–3.92	4.50	3.64–5.36	0.0001	significant
Cl <sup>k</sup> , mmol/l	112.0	105.4–118.0	118.0	112.2–124.4	0.0001	significant
BUN <sup>m</sup> , mmol/l (mg/dl)	22.5 (63.0)	15.0–33.4 (42.0–93.6)	28.57 (80.0)	10.00–52.07 (28.00–145.80)	0.0457	
UA <sup>n</sup> , mmol/l (mg/dl)	0.02 (0.50)	0.01–0.06 (0.30–1.06)	0.04 (0.80)	0.02–0.20 (0.46–3.40)	0.0002	
Glucose, mmol/l (mg/dl)	4.39 (79.00)	3.04–5.79 (54.80–104.20)	5.53 (99.50)	4.47–9.14 (80.50–164.50)	0.0004	significant
Total protein, g/l (g/dl)	36.0 (3.6)	28.8–46.0 (2.88–4.60)	40.00 (4.00)	30.40–51.40 (3.04–5.14)	0.0812	
Albumin, g/l (g/dl)	13.0 (1.3)	10.0–16.0 (1–1.6)	11.0 (1.1)	9.0–14.0 (0.9–1.4)	0.026	
Globulin, g/l (g/dl)	22.2 (2.2)	17.40–31.80 (1.74–3.18)	28.0 (2.8)	20.80–39.80 (2.08–3.98)	0.0052	
ALP <sup>r</sup> , IU/L	9.0	6.4–17.6	23.0	13.8–67.4	0.0001	significant
Mg <sup>s</sup> , mmol/l (mg/dl)	2.17 (5.00)	1.71–2.83 (3.94–6.52)	2.26 (5.20)	1.75–2.77 (4.02–6.38)	0.5999	
LDH <sup>t</sup> , IU/L	120.0	51.0–323.0	227.0	89.0–453.0	0.0123	
AGAP	10.8	6.06–20.02	17.5	12.84–28.04	0.0001	significant

<sup>a</sup> W/L<sup>3</sup>, weight/length<sup>3</sup>; <sup>b</sup> RBC, red blood cell count; <sup>c</sup> WBC, white blood cell count; <sup>d</sup> HCT, hematocrit; <sup>e</sup> HET, heterophils; <sup>f</sup> Ca, calcium; <sup>g</sup> P, phosphorus; <sup>h</sup> CP, calcium/phosphorus ratio; <sup>i</sup> AST, aspartate aminotransferase; <sup>j</sup> Na, sodium; <sup>k</sup> K, potassium; <sup>l</sup> Cl, chloride; <sup>m</sup> BUN, blood urea nitrogen; <sup>n</sup> UA, uric acid; <sup>o</sup> ALP, alkaline phosphatase; <sup>p</sup> Mg, magnesium; <sup>q</sup> LDH, lactate dehydrogenase; <sup>r</sup> AGAP, Anion Gap also known as AG.  
\* GGT, gamma glutamyltransferase, was consistently below measurable levels.

knowns in the residential group. Thirty-three percent of the resident turtles and 10% of the migrating animals had recorded lesions, which were defined as full-thickness dermal abrasions or lacerations on the shell or skin.

### Body condition and hematologic parameters

There was no correlation between body condition indexes (weight, W/L ratio, and Fulton's K ratio) and hematologic patterns. Weight and HCT did have a positive association.

### Epibiota

The barnacle *Chelonibia testudinaria* occurred more frequently than any other epibiont. *C. testudinaria* was found on the flippers, plastron, and head of some turtles, but these barnacles were not included in the epibiota assessment. Other organisms found on the turtles' carapaces included: Atlantic ribbed mussel (*Geukensia demissa*), Ivory barnacles (*Balanus eburneus*), amphipods (*Carporella* sp.), bryozoa (*Bugula neritina*), Eastern oyster (*Crassostrea virginica*), unspiciated tunicates, polychaete worms (tubed), green algae, and red algae. *C. testudinaria* accumulation ranged from 6 to 386 individuals on the migratory turtles, with a median number of 146 (21–344) ( $n = 18$ ). Barnacle load between the two groups was significantly different (Wilcoxon rank sum test,  $P < 0.001$ ) on the residential group ( $n = 15$ ) ranging from 97–3,667 with a median of 591 (113–3452).

### Red blood cell processing (hematocrit and red blood cell size)

Red cell surface area was  $3.13 \mu\text{m}^2$  (minimum  $2.88 \mu\text{m}^2$ , maximum  $3.39 \mu\text{m}^2$ ) for the resident population and  $2.90 \mu\text{m}^2$  (minimum  $2.58 \mu\text{m}^2$ , maximum  $3.23 \mu\text{m}^2$ ) for the migratory population. No statistical difference between the two populations was found. No statistical difference was found with the blood sample run in the centrifuges at variable speeds and times.

### Barnacle load versus hematologic parameters

Percent barnacle coverage did not correlate with any clinical pathology analyte (Kendall-tau,  $P > 0.05$ ). The two surface area measurement programs Sigma-Scan<sup>TM</sup> software (used for migratory turtles) and the Optima software (used for resident turtles) provided reasonably similar mean areas with variation between methods of 0.3–4.3%.

### Residential and migratory group patterns

Thirty-three comparisons of resident and migratory groups were performed by the Wilcoxon rank

sum test (Table 1). Of these, twenty-four had  $P$  values  $< 0.05$  (weight, SCL, HCT, total RBC (TRBC), % HET, % LYMPH, LYMPH, EOS, % EOS, AZURO, Ca, P, Ca/P, AST, LDH, Na, K, Cl, GLU, ALP, BUN, UA, ALB, GLOB and AGAP). To reduce type I errors, a sequential Bonferroni test was performed.<sup>12</sup> The sequential Bonferroni test indicated fourteen parameters that should be considered significantly different between turtle populations ( $P < 0.0015$ ). These included TRBC, % HET, % LYMPH, LYMPH, EOS, Ca, AST, UA, Na, K, Cl, GLU, ALP, and AGAP.

## DISCUSSION

The data on health parameters examined in this study were more variable than expected; this was most likely because of a relatively small sample size despite the large capture effort (migratory turtles = 9504 net.hr; resident turtles = 2304 net.hr). One complicating factor may have been the possible capture of residential turtles during the migratory time. Four out of forty-two turtles (10%) captured in the fall were also captured during summer months within 5 yr of the project. These animals were retained in the migratory group for analysis purposes because 1) they were sampled in the same environmental conditions, 2) they are thought to be starting a shorter migration themselves at that time, 3) there are no objective criteria to exclude other Pamlico resident juveniles that simply avoided summer capture, and 4) their physiologic parameters were not outliers within the migratory group.

Medians for weight, length, WBC, HCT, LYMPH, EOS, CA, AST, Na, K, Cl, BUN, GLU, TP, GLOB and AGAP levels were greater in the residential versus the migrating turtles. The higher TP, GLU, BUN, Na, K, and Cl are indicative of feeding animals.<sup>4</sup> Elevated WBC with increased LYMPH, GLOB, and EOS levels may indicate antigenic stimulation. The elevated AST may have indicated some mild tissue damage, which was observed by the authors in a higher percentage of summer resident turtles showing full-thickness dermal and shell lesions (33% of resident turtles versus only 10% of the migrating animals). There was no indication that the apparent increase in lesions in the summer resident group correlated with any clinically important change.

Migratory turtles had a higher percentage of heterophils and a lower lymphocyte and eosinophil count. This could be interpreted as a "stress leukogram," supporting a stress hypothesis in migratory animals. This response by reptiles is not well documented in the literature, but increased cortisol levels have been published in migrating shorebirds



and salmon.<sup>3,11</sup> Increased cortisol (corticosterone in sea turtles) can lead to secondary shifts in other hematologic parameters. Another source of stress could have been handling, but both the migratory and summer resident turtles were accessed using the same protocols and all blood samples were taken within 15 min of capture.

The migrating turtles had decreases in sodium, potassium, calcium, and chloride, which may have been caused by the lack of feeding during this time. The major route of salt intake for loggerhead sea turtles is from incidental saltwater intake during feeding and ingestion of invertebrates, which have up to three times as much salt as sea turtle body fluids (a relative fasting state was also supported by the lower BUN and GLU parameters found in the migrating turtles). The anorexic state of migratory animals may not permit compensation for losses through the salt gland, intestine, and kidney.<sup>6</sup>

There was no statistically significant association between body condition and any of the hematologic parameters. However, even with the irregular distribution, nonparametric test of association indicated a significant correlation between HCT and weight. The fact that the small sample size detected this positive correlation between HCT and body condition indicated that either of these parameters might be able to be utilized as an indicator of fitness. This association could be caused by larger turtles diving for longer periods of time. The contradictory results of the TRBC numbers (migratory =  $0.88 \times 10^6/\text{mm}^3$ , resident =  $0.38 \times 10^6/\text{mm}^3$ ) and HCT (migratory = 28, resident = 32) when comparing the resident and migratory groups were counterintuitive. An inverse relationship between the TRBC and the size of the erythrocytes between species has been reported, but it is unclear whether this occurs within the same species.<sup>1</sup> TRBC also changes with the environmental conditions, nutritional status, sex, and season.<sup>1</sup> Another factor that may increase TRBC in migrating animals is that they spend more time swimming, thus requiring increased oxygen consumption. One study found that swimming juvenile green turtles increased their oxygen consumption needs by 10-fold over resting metabolic rates, indicating a heavy reliance on aerobic metabolism during routine swimming.<sup>10</sup> Migrating animals may need a higher TRBC to compensate for the increased metabolic needs of swimming. Analysis of RBC size did not show a significant difference between the two populations, and variable centrifuge speeds and times applied to the same blood sample of six sea turtle blood samples did not provide significant variations with HCT. This would suggest that the manual counting of

Table 2. Epibiota: Barnacle percent coverage.

Percent coverage	Migration (n = 18)	Resident (n = 15)
Median	13.02	10.75
10th percentile	0.39	4.24
90th percentile	29.09	28.23

RBCs by different technicians may be the source of this difference.

Epibiotic load has been considered a possible indicator of sea turtle health and has been included as one of several criteria in a recent in-water health assessment in South Carolina and Georgia.<sup>7</sup> The percent coverage of epibiota was calculated using the two methods. The two methods were compared and gave acceptably similar results with a -0.4 to 4.3% discrepancy. The Optimas system gave a slightly higher surface area, most likely because its zooming capabilities allowing for better barnacle outlines. This may have resulted in finer detail, thus allowing capture of some of the smaller barnacles and the surface area between them. In both the summer and fall capture groups, the barnacle *Chelonibia testudinaria* occurred more frequently than any other epibiont. It is interesting to note that the large discrepancy of the number of barnacles per turtle did not necessarily translate to an increased percent coverage (Table 2). Smaller-sized barnacles were found on turtles with high populations of epibiota. The cause of this discrepancy between the two groups is unknown, but it may be because of variation in each turtle's historical environmental exposure and the resident turtles' possibly having a more sedentary lifestyle. There was no correlation between barnacle load and any hematologic parameters. This may have been an artifact of the small sample size, or barnacle loads may not have been extreme enough to generate health impacts observable through examination of the parameters we explored. This study was limited to the shell because of the complexity surrounding a standardized method of analysis. Surveying other body parts, such as flippers, was not done in the study, but future studies may examine this and may yield different results.

The comparative hematologic and biochemical findings of the migratory animals versus the resident animals are consistent with findings about other animals that are not eating and under physiologic stress. The epibiota findings were counter to the authors' initial hypothesis that epibiota would be an indicator of ill thrift. The fact that turtles in various health states within the study did not show

heavy epibiotic loads when compared to healthy turtles indicates that epibiota is not likely a sensitive indicator of turtle health but the low sample size clearly indicates the need for future studies. The statistical differences of the impact of epibiota and migration found in this study may not have clinical significance to the individual turtle, but they do indicate the differences in the characteristics of these two populations sampled and highlight the complexities of trying to establish reference ranges for animals under varying conditions. These differences need to be considered when examining populations and considering evaluation of animals during various stages of their life histories.

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